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A B S T R A C T

Objective: The aim of this study was to investigate the effects of dietary silicon-enriched spirulina (SES) on atherosclerosis.
Methods: Hamsters (six per group) on a high-fat (HF) diet received SES or non-enriched spirulina (both at 57 mg/kg body weight) daily. This corresponded to 0.57 mg silicon/kg body weight daily.
Results: The HF diet induced dyslipidemia, insulin resistance, oxidative stress, and vascular dysfunction. Compared with the HF group, SES attenuated increases of lipemia and prevented insulin resistance (IR) ($P = 0.001$). SES protected against oxidative stress through a reduction of heart ($P = 0.006$) and liver ($P < 0.0001$) nicotinamide adenine dinucleotide phosphate-oxidase activity and by sparing the activity of superoxide dismutase ($P = 0.0017$) and glutathione peroxidase ($P = 0.01861$). SES decreased inflammation, lowering tumor necrosis factor-α ($P = 0.0006$) and interleukin-6 levels ($P = 0.0112$), decreasing polymorphonuclear cells and preventing nuclear factor-κB activity ($P = 0.0259$). SES corrected plasma level of monocyte chemoattractant protein-1 ($P = 0.0380$), which was increased by the HF diet. Finally, SES supplementation prevented vascular and endothelial functions assessed respectively by the contractile response to the agonist phenylephrine and the relaxation induced by acetylcholine.
Conclusion: SES protects against metabolic imbalance, inflammation, oxidative stress, and vascular dysfunction induced by an HF diet, and could prevent the atherogenic processes. Synergistic effects between spirulina and silicon were observed.

Introduction

The cyanobacteria *Spirulina platensis* is commercially available for human consumption. It represents one of the richest protein sources of plant origin (60–70%), lipids (7%), and carbohydrates (20%) and is a good source of vitamins and minerals such as calcium, magnesium, phosphorus, potassium, sodium, and zinc [1]. This microalga is one of the most potent nutrient sources and is used as a nutraceutical food supplement [2] with no toxic side effects [3], although there is insufficient scientific evidence to recommend supplementation in humans. Spirulina are particularly suitable for the production of specific bioactive compounds and nutritional elements that they are able to accumulate in an organically biotransformed form. Several reports have described successful enrichment of spirulina biomass in selenium [4], iron, or chromium. Thus, a new type of food supplement has been developed and could serve as a rich source of trace elements [5]. As previously reported, ample evidence

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exists to indicate that silicon may be an essential nutrient for higher animals, including humans [6].

Silicon is naturally found in insoluble but not bioavailable forms. The main soluble form is orthosilicic acid $Si(OH)_4$, which tends to polymerize at high concentration (>2 mM Si), corresponding to the form absorbed by humans either in drinking water and food or appearing after nutrient hydrolysis in the intestine [7]. Although real dietary deficiencies are unknown, a lack of food diversification and low consumption of fruits and vegetables could result in insufficient coverage of silicon needs. Generally, the main food sources of silicon are bananas, cereal, and beer [8,9]. In humans, the daily intake is about 20 to 50 mg, and the body needs are 9 to 14 mg/d [10,11].

The biological importance of silicon has to be considered in the context of its body distribution. The highest concentrations are found in bone and connective tissues, such as the aorta, trachea, tendons, and skin, where silicon appears to act on extracellular matrix turnover via collagen and elastin synthesis [6]. Silicon supplementation has been reported to have beneficial effects on these tissues, especially bones [12] and skin [13]. In contrast, silicon deficiency has been associated with detrimental effects on bone mineralization and growth [14] and skin elasticity and healing [6]. The importance of silicon also has been demonstrated in cardiovascular pathophysiology, especially in the prevention of atherosclerosis [15]. Studies have shown an inverse relationship between the ingestion of silicon and the development of atherosclerosis [16,17]. Furthermore, silicon supplementation reduces hypertension and increases antihypertensive and antiatherogenic gene expression in the aorta of spontaneously hypertensive rats [18].

Incorporating silicon into spirulina could be a way to produce a bioavailable food supplement. Thus, in line with the beneficial effects of silicon and the antioxidant, hypolipidemic, and anti-inflammatory properties of spirulina [19], we focused on a model of early atherosclerosis to evaluate the effects of supplementation with silicon-enriched spirulina (SES). We assessed the potential beneficial preventive effects of SES on some major disorders and dysfunctions induced by a high-fat (HF) diet in the Syrian hamster model.

Materials and methods

Animals, diets, and experimental design

Production of the materials

To produce SES, spirulina (PhycoBiotech, Lunel, France) was grown in a 130 L photobioreactor under continuous lighting on Zarouk's medium at 22°C and pH 10.5 in the presence of 1 g/L sodium metasilicate (Na_2O_3Si). This medium contained $NaHCO_3$, 16.8 g/L; K_2HPO_4, 0.5 g/L; $NaNO_3$, 2.5 g/L; K_2SO_4, 1.0 g/L; NaCl, 1.0 g/L; $MgSO_4.7H_20$, 0.2 g/L; $CaCl_3$, 0.04 g/L; $FeSO_4.7H_20$, 0.01 g/L; EDTA, 0.08 g/L; H_3BO_3, 2.86 mg/L; $MnCl_2.4H_2O$, 220 mg/L; $CuSO_4.5H_2O$, 79 mg/L; MoO_3, 15 mg/L; and Na_2MoO_4, 21 mg/L, and was supplied with light aeration (30 L/min) and the addition of 0.03% CO_2. At the end of the growth, the biomass was recovered and filtered through a 20-mm membrane, thoroughly washed with distilled water, frozen, and lyophilized. Resultant SES contained 1% silicon, whereas regular spirulina contained <0.023% silicon (lower detection limit), as indicated by the manufacturer.

Animal husbandry/maintenance

Twenty-four male weanling Golden Syrian hamsters (Janvier-Labs, Le Genest-St-Isle, France), weighing ~90 g each, were randomly divided into four groups of six animals (six animals per plastic cage). They were housed at 23 ± 1°C, subjected to a 12-h light/dark cycle and handled in compliance with European Union rules and according to the guidelines of the National Institutes of Health [20] and the Committee for Animal Care at the University of Montpellier (France) (permission number C 34 249).

Animal protocol design

Three groups were fed an HF atherogenic diet (HFD), consisting of 200 g/kg casein, 3 g/kg L-methionine, 393 g/kg corn starch, 154 g/kg sucrose, 50 g/kg cellulose, 100 g/kg hydrogenated coconut oil, 2 g/kg cholesterol, 35 g/kg mineral mix, and 10 mg/kg vitamin mix, for 12 wk. For reference, a fourth group received a standard diet (STD) consisting of 236 g/kg casein, 3.5 g/kg L-methionine, 300 g/kg corn starch, 30 g/kg maltodextrin 10, 290.5 g/kg sucrose, 50 g/kg cellulose, 45 g/kg vegetable oil, 35 g/kg mineral mix, and 10 g/kg vitamin mix. In both diets, mineral and vitamin mixes were formulated according to American Institute of Nutrition-93 guidelines [21]. The five groups had free access to both food and tap water, which contained <0.023% silicon (lower detection limit).

The hamsters of each group received daily by gavage 1 mL of either tap water (STD and HFD groups), or crude spirulina (Sp) suspended in tap water at 57 mg/kg body weight (HF-Sp group), or SES suspended in tap water at 57 mg/kg body weight (HF-SES group). Spirulina concentration was determined according to a previous study [22] and its silicon content (1%), and corresponded to a daily intake of 40 mg for a 70 kg-human [23].

Analytical procedures

Plasma analysis

At the end of the experimental period, fasting blood samples were collected by cardiac puncture. Plasma was prepared by centrifugation at 2000*g* for 10 min. Total cholesterol and high-density lipoprotein cholesterol (HDL-C) were determined in plasma (10 μL) using enzymatic kits (Randox Laboratories Ltd, Crumlin, UK) according to the manufacturer; HDL-C was measured after precipitation of very low- and low-density lipoprotein cholesterol (LDL-C) using phosphotungstate reagent. Triacylglycerols and glucose levels were measured in plasma (10 μL) using a Randox enzymatic kit and reagents from the Thermo Electron Corporation (Cergy Pontoise, France), respectively. Paraoxonase activity (PON) was determined using paraoxon as a substrate and measured by the increase in absorbance at 412 nm, as previously described [24]. Insulinemia (10 μL plasma) was determined using enzyme-linked immunosorbent assay (ELISA) kits (Mercodia AB, Uppsala, Sweden). Plasma monocyte chemoattractant protein (MCP)-1 concentration was measured using specific ELISA kit according to the manufacturer (R&D Systems, Europe, Lille, France) and 100-μL plasma.

The homeostatic model assessment for insulin resistance (HOMA-IR) was determined from fasting insulin and glucose values as previously described [25] and according to:

$$\text{HOMA} - \text{IR} = \frac{\left[\text{fasting glucose}\left(\text{mmol}/\text{L}\right) \times \text{fasting insulin}\left(\text{mU}/\text{L}\right)\right]}{22.5}$$

Liver anatomo-pathology

The liver was excised; some samples were removed for histology, whereas others were stored at −80°C until further use. For pathologic analysis, liver samples were fixed in 10% neutral-buffered formaldehyde and paraffin embedded, and 3 μm-thick serial sections prepared. Sections were deparaffinized and stained with hematoxylin and eosin.

Liver antioxidant enzymes activity

Unfixed liver samples were homogenized (5%, w/v) in ice-cold 0.1 mol/L potassium phosphate buffer (pH 7.4) and the homogenate was spun at 13 000*g* for 15 min at 4°C. The supernatant was then stored at −80°C for subsequent assay of superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity using commercial kits (Randox Laboratories LTD, Crumlin, UK), and to quantify liver tumor necrosis factor (TNF)-α and interleukin (IL)-6 levels using specific ELISA kits according to the manufacturer's instructions (R&D Systems Europe, Lille, France).

Liver inflammation

To assay nuclear factor (NF)-κB activity, liver tissue was homogenized (7.5% w/v) in a hypotonic buffer (pH 7.9) containing 20 mM HEPES, 10 mM EDTA, 10 mM KCl, 1% protease inhibitor cocktail, 0.1% dithiothreitol, and 0.1% Igepal. Nuclear extracts were then obtained by homogenizing the pellet in lysis buffer (pH 7.9) containing 20 mM HEPES, 1 mM EDTA, 200 mM NaCl, 10% glycerol, 1 mM dithiothreitol, and 1% protease inhibitor cocktail. NF-κB activity was determined from nuclear extracts using a commercial immunoassay kit (Active Motif, Rixensart, Belgium) and according to manufacturer's instructions.

Liver and cardiac oxidative stress

Hepatic and cardiac superoxide anion ($O2^{\circ -}$) production was evaluated by the intensity of lucigenin-enhanced chemiluminescence (10 μM lucigenin) as previously described [24]. Results were expressed as relative luminescence units/mg of protein.

Vascular reactivity

The thoracic aorta was used to study ex vivo the responses to agonists or antagonists of arterial contraction. Immediately after removal, arterial tissue was immersed in phosphate saline buffer, pH 7.4 containing (in mM) 140 NaCl, 5 KCl, 1 $MgCl_2$, 0.5 KH_2PO_4, Na_2HPO_4, 2.5 $CaCl_2$, 10 HEPES, and 10 glucose. Aortic tissue was cleaned of fat and connective tissue and cut into 2- to 3-mm–wide rings. Aortic rings were mounted in standard organ bath chambers (EMKA Technologies, Paris, France), maintained at 37°C and continuously bubbled with O_2, then changes in isometric tension were recorded as previously described [25]. Each arterial segment was subjected to a 60-min equilibration period at the predetermined optimal basal tension of 1 g. The contractile function of each segment was assessed with 1 μM phenylephrine (PE) and the presence of endothelium was confirmed by the vasorelaxation induced after application of acetylcholine (Ach; 1 μM). After several washouts and a 20- to 30-min period of stabilization, dose responses were performed by cumulative increases in the concentration of the agonist PE (0.01–10 μM range). Endothelial function was assessed by studying the relaxing effects of cumulative increases in the concentration of ACh (1 nM–10 μM) in arteries contracted with a submaximally active concentration of PE (10 μM). Endothelium-independent relaxations to sodium nitroprusside (SNP; 1 nM–100 μM) were studied in endothelium-denuded rings previously contracted with PE (10 μM). Each protocol was performed in triplicate in tissue from six different animals per group.

Statistical analysis

All data were expressed as mean ± SEM. One-way analysis of variance was followed by a multiple comparisons Student-Newman-Keuls post hoc test was used to compare differences between groups. Differences were considered statistically significant at $P < 0.05$.

Results

Food intake and body weight

The HF diet significantly increased the food intake by 27% ($P = 0.0062$) and the gain in body weight by ~80% ($P < 0.001$) of hamsters in the HF group compared with the STD group (Table 1). HF-SES and HF-Sp groups showed significantly less weight gain than the control HF group, that is, 52% ($P = 0.0001$) and 44% ($P = 0.0008$), respectively, although there were no differences in food intake (Table 1).

Plasma analysis

Hamsters fed HF diet showed dyslipidemia compared with those in the STD group (Table 1). Except for LDL-C, supplementation with SES improved these parameters, whereas spirulina did not.

Compared with the STD group, the HF group showed a 40% higher fasting plasma glucose level ($P = 0.0256$) and a 194% increase in insulinemia ($P < 0.0002$), leading to a 126% increase in insulin resistance (IR) as measured by HOMA-IR ($P = 0.0075$; Table 1). Although in HF-SES glycemia was elevated, plasma insulin level (72%, $P < 0.0001$) and HOMA-IR (56%, $P = 0.0010$) were identical to those of the STD group. These parameters the same between the HF and HF-Sp groups.

Plasma PON activity is summarized in Table 1. Its activity was reduced by about 32% in the control HF group compared with the STD group ($P = 0.001$), which did not occur in groups HF-SES and HF-Sp.

In HF-fed hamsters, MCP-1 plasma levels were raised by 420% ($P < 0.0001$) compared with the STD group (Table 1). The level was lowered by 28% in HF-SES group ($P = 0.0380$), whereas HF-Sp showed a weaker effect compared with the HF group.

Liver and cardiac oxidative stress

In the liver, $O_2^{\circ-}$ production (i.e. NADPH oxidase activity) increased by 98% ($P < 0.0001$) in the HF group when compared with STD group. This production was reduced in the HF-SES and

Table 1
Nutritional, plasma, and liver Biochemical parameters in the different Groups*

	STD	HF	HF-SES	HF-Sp
Body weight gain (g)	32.7 ± 5.2[a]	58.7 ± 7.2[b]	28.6 ± 4.3[a]	33.0 ± 2.8[a]
Food intake (g/d)	4.9 ± 0.2[a]	6.2 ± 0.6[b]	5.5 ± 0.6[b]	5.5 ± 0.5[b]
Plasma				
TC (mmol/L)	3.19 ± 0.15[a]	7.26 ± 0.26[b]	6.11 ± 0.37[c]	6.81 ± 0.23[b]
HDL-C (mmol/L)	1.97 ± 0.23[a]	3.26 ± 0.18[b]	2.52 ± 0.16[c]	3.03 ± 0.12[b]
LDL-C (mmol/L)	1.22 ± 0.23[a]	4.00 ± 0.32[b]	3.59 ± 0.37[b]	3.78 ± 0.24[b]
TG (mmol/L)	1.06 ± 0.06[a]	1.73 ± 0.27[b]	1.14 ± 0.03[a]	1.60 ± 0.29[ab]
PON (U/mL)	172.8 ± 10.2[a]	118.2 ± 11.6[b]	146.9 ± 9.9[ab]	151.0 ± 7.7[a]
Glucose (mmol/L)	7.78 ± 0.13[a]	10.88 ± 0.67[b]	12.28 ± 0.91[b]	12.04 ± 1.46[b]
Insulin (pmol/L)	155.4 ± 43.1[a]	456.7 ± 79.8[b]	127.8 ± 43.9[a]	178.0 ± 30.5[a]
HOMA-IR	9.98 ± 2.90[a]	22.55 ± 4.90[b]	9.92 ± 0.80[a]	12.06 ± 2.53[c]
MCP-1 (pg/mL)†	30.5 ± 11.5[a]	59.5 ± 19.4[b]	114.2 ± 7.7[c]	133.0 ± 22.4[bc]
Liver				
Inflammation				
TNF-α (pg/mg protein)	52.1 ± 7.9[a]	122.8 ± 14.6[b]	63.0 ± 7.7[a]	102.9 ± 19.2[b]
IL-6 (pg/mg protein)	220.7 ± 14.7[a]	362.6 ± 22.2[b]	254.3 ± 31.2[a]	344.5 ± 30.2[b]
NF-κB (ng/g liver)	6.2 ± 0.5[a]	8.5 ± 0.5[b]	6.7 ± 0.4[ac]	8.2 ± 0.4[bc]
Oxidative status				
$O_2^{\circ-}$(RLU/mg protein)‡	55.36 ± 3.95[ac]	109.60 ± 5.74[b]	39.94 ± 5.31[a]	63.04 ± 4.27[c]
Cardiac oxidative status				
$O_2^{\circ-}$ (RLU/mg protein)‡	61.98 ± 4.16[a]	124.18 ± 9.58[b]	81.25 ± 5.94[c]	99.33 ± 5.10[d]
Antioxidant enzyme activity				
SOD (U/mg protein)	39.8 ± 4.1[a]	2.8 ± 3.2[b]	34.3 ± 6.3[a]	23.5 ± 0.4[a]
GPx (U/mg protein x 10^{-2})	79.1 ± 5.6[a]	138.6 ± 9.8[b]	107.5 ± 5.8[c]	102.6 ± 9.1[ac]

GPx, glutathione peroxidase; HDL-C, high-density lipoprotein cholesterol; HF, high fat; HOMA-IR, homeostatic model assessment for insulin resistance; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; MCP, monocyte chemoattractant protein; NF, nuclear factor; PON, paraoxonase activity; RLU, relative luminescence unit; SES, silicon-enriched spirulina; SOD, superoxide dismutase; Sp, spirulina; STD, standard diet; TC, total cholesterol; TG, triacylglycerol; TNF, tumor necrosis factor

* Values are means ± SEM (n = 6). For each dietary treatment, means in a row with different letters differ, $P < 0.05$. Plasma TC, HDL-C, LDL-C, TG, PON, and HOMA-IR were measured after overnight fasting, at the end of the 12-wk treatment period.

† Plasma MCP-1 levels.

‡ $O_2^{\circ-}$ production (i.e., NADPH oxidase activity, expressed as RLUs).

HF-Sp groups (64%; $P < 0.0001$ and 42%; $P < 0.0001$, respectively) compared with the HF group (Table 1).

In the left cardiac ventricle, $O_2^{\circ -}$ production increased by 101% ($P < 0.0001$) in the HF group compared with the STD group. It was reduced by 35% ($P = 0.0060$) and 20% ($P < 0.0001$) in hamsters receiving HF-SES and HF-Sp, respectively, compared with the HF group (Table 1).

Liver antioxidant enzymes activity

In the HF group, the activities of liver SOD and GPx increased by 35% ($P = 0.0171$) and 75% ($P < 0.0001$), respectively, compared with the STD group (Table 1). In HF-SES and HF-Sp groups, the SOD activity fell by 35% ($P = 0.0017$) and 55% ($P < 0.0001$), respectively, and the activity of GPx dropped by 23% ($P = 0.01861$) and 24% ($P = 0.0049$), respectively (Table 1). Additionally, in these two groups, the level of SOD activity corresponded to that measured in the STD group.

Liver steatosis and inflammation

STD hamsters showed no histologic evidence of hepatic steatosis (Fig. 1). In contrast, the presence of severe intensity microvascular steatosis appeared in HF group, accompanied by a low inflammatory reaction as revealed by the presence of polymorphonuclear (PMN) cells. No marked reduction in the degree of steatosis appeared in the liver of the HF-SES and HF-Sp groups, although some PMN cells could be observed at a lesser extent.

The concentrations of TNF-α and IL-6 in the livers of HF group were significantly increased by 135% ($P < 0.0001$) and 64% ($P = 0.0015$), respectively, compared with the STD group (Table 1). This low-grade inflammatory state was prevented only in the HF-SES group, which showed cytokine levels similar to those of the STD group.

In the HF group, NF-κB activity increased (36%, $P = 0.0051$) compared with the STD group. In the HF-SES group, such an increase was prevented but not in Hf-Sp, and the hepatic NF-κB activity (21%, $P = 0.0259$) reached the level of the STD group (Table 1).

Vascular reactivity

Atherosclerosis induced functional alterations in the vasomotor properties of blood vessels affecting both vascular endothelium and smooth muscle. In our model, the HFD induced such modifications in the vasomotor responses of hamster aorta. The contraction induced by PE was lower in the HF group (2.57 ± 0.13 g) than in the STD animals (3.16 ± 0.19 g; Fig. 2A), corresponding to a 39% reduction in the contractile response ($P = 0.0477$). This reduction was not observed in the HF-SES group (3.38 ± 0.15 g), for which the response to PE was identical to that of STD group ($P = 0.344$ versus STD and $P = 0.0005$ versus HF). The vasorelaxant properties of arteries were also

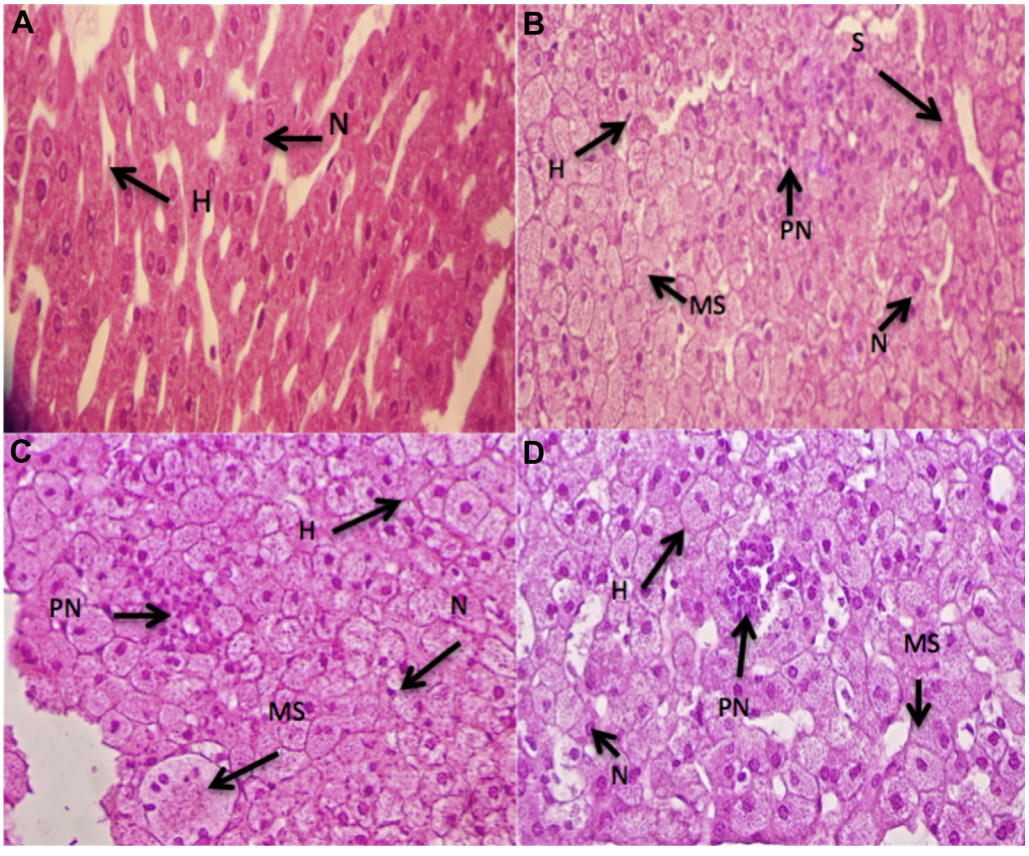

Fig. 1. Histologic evaluation of hepatic steatosis in STD (A), HF (B), HF-SES (C), and HF-Sp (D) groups. Images illustrated represent liver sections (40 × magnification) and showed moderate to severe hepatic steatosis in HF animals (B) with microvascular steatosis (MS), sinusoidal capillaries (S) and inflammation indicated by the widespread presence of polynuclear cells (PN), compared with STD animals (A). Hepatic steatosis is not prevented by Sp (C) and SES (D) supplementations, although PN cells number is lowered. HF, high fat; SES, silicon-enriched spirulina; Sp, spirulina; STD, standard.

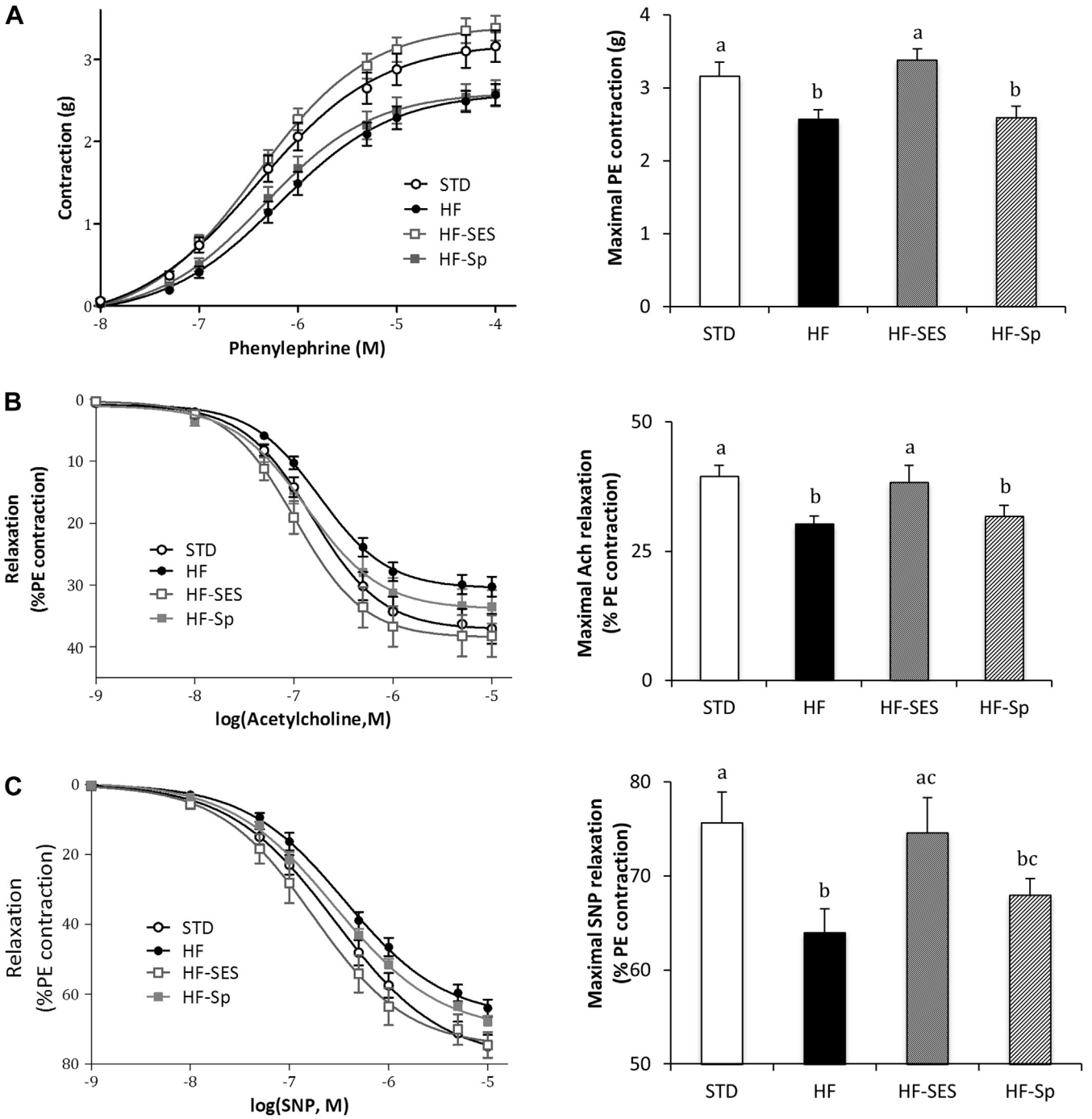

Fig. 2. Vascular reactivity of hamster aorta in STD, HF, HF-SES, and HF-Sp groups. (A) Contractility was evaluated in response to cumulative concentrations of PE. Endothelium-dependent relaxation in response to cumulative doses of Ach (B) and smooth muscle–dependent relaxation in response to SNP (C) were studied on arteries previously contracted with PE (10 μM). Left panels: Dose-response curves. Right panels: Maximal contraction or relaxation obtained for each group in various conditions. Values are means ± SEM (n = 6). Ach, acetylcholine; HF, high fat; PE, phenylephrine; SES, silicon-enriched spirulina; SNP, sodium nitroprusside; Sp, spirulina; STD, standard.

altered in HFD group. Cumulative addition of Ach and SNP resulted in a concentration dependent relaxation of aorta previously contracted with PE (Fig. 2B, C). The HFD group showed reduced endothelium-dependent response to Ach compared with the STD group (Fig. 2B). The maximal relaxation obtained for the HFD group was reduced by 23% compared with the Ach response in the STD group ($P = 0.0042$). This reduction was observed in the HF-Sp group (19%, $P = 0.0334$) but not in the HF-SES group, in which the relaxation to Ach was identical to that of the STD group ($P = 0.396$). In the same way, SNP induced a dose-dependent relaxation of rat aorta previously contracted with PE. In the HF group, the relaxing effect of SNP was lower than in the STD group, relaxation was reduced by 15% ($P = 0.0218$) compared with the STD group. In the HF-SES group, the relaxation to SNP was identical to that of the STD group ($P = 0.974$), whereas in the HF-Sp group, no difference was

observed in the HFD group ($P = 0.338$). The HFD induced vascular and endothelial dysfunctions, which were completely prevented by SES supplementation.

Discussion

We investigated the effects of SES on the disorders induced by an HFD and associated with early atherosclerosis in hamsters. We showed that supplementation with SES prevents disorders including dyslipidemia, oxidative stress, inflammation, IR, and vascular dysfunction. Although some improvements in dyslipidemia, oxidative stress, and inflammation could be attributed to spirulina, per se [26], the prevention of IR and vascular dysfunction more likely reflects an additional effect of silicon. Our results specifically corroborate the relevance of spirulina enrichment with silicon and highlight the efficacy of such food supplement in the prevention of cardiovascular risk factors.

As expected, the HFD, characterized by high levels of saturated fatty acids and cholesterol, induced dyslipidemia with increased triacylglycerols, HDL-C, and LDL-C. This was partially prevented by spirulina, whether or not enriched. The HFD, promoting high plasma glucose and insulin, also induced IR, as revealed by the HOMA-IR. Although supplementation with SES did not avoid the increase in glycemia, IR was prevented due to reduced insulinemia. The decrease in insulin sensitivity induced by the HFD is generally associated with other disturbances such as oxidative stress and low-grade inflammation, both of which contribute to the development of atherosclerosis [27]. In our model, the HFD did indeed induced cardiac and hepatic oxidative stress, as evidenced by $O_2^{\circ -}$ overproduction. The NADPH oxidase system is a major source of $O_2^{\circ -}$ production and any increase in its activity leads to an oxidative stress as shown before with increased activity and expression of p22 ^{phox} NADPH oxidase subunit [28].

In the present study and as shown in a previous study [19], both crude spirulina and SES improved oxidative status in the liver and the heart by decreasing $O_2^{\circ -}$ production. Generally, reactive oxygen species (ROS) overproduction is counteracted by induction of the antioxidant defense system to restore redox homeostasis, which includes SOD, GPx and catalase activities indeed increased by HF diets [29]. Here, in the HFD group, hepatic SOD and GPx activities were enhanced. However, this induction did not prevent hepatic $O_2^{\circ -}$ overproduction, suggesting that ROS production could overwhelm enzymatic defense mechanisms, this imbalance resulting in liver oxidative stress.

In hamsters fed with either crude spirulina or SES, GPx and SOD activities were lower than in HFD animals. Therefore, in these groups, the decreases in hepatic and cardiac $O_2^{\circ -}$ production are not related to an increase in enzymatic antioxidant defenses, but rather to a sparing effect due to their direct antioxidant properties [30]. This has been reported in other studies [24,31] where dietary antioxidants (phenolics) consumption decreased endogenous antioxidant enzymes, scavenging oxygen radicals and reducing the requirement for cellular antioxidant defense.

PON activity is another marker of oxidative stress. PON plays a major role in the antioxidant activity of HDL [32]. It is able to hydrolyze oxidized phospholipids and lipid peroxidation products, thereby inhibiting LDL and HDL oxidation. Thus, PON could protect against atherosclerosis, and modulation of its activity is a part of current therapeutic targets [33]. HF diets associated with oxidative stress is known to decrease PON activity [34] and herein, this activity was strongly reduced in the HFD group.

It is well known that IR associated with hepatic oxidative stress is related to the development of nonalcoholic fatty liver disease (NAFLD), which is presented as an independent risk factor for atherosclerosis and cardiovascular disease, although the mechanistic relationships are not fully understood [35]. We detected liver steatosis in the HFD group, as reflected by lipid accumulation in the tissue. Despite the presence of PMN cells, no sign of fibrosis was detected in the liver, suggesting that the stage of nonalcoholic steatohepatitis (NASH) was not yet reached. The low-grade inflammation state is confirmed by cytokine levels (TNF-α and IL-6) that were increased compared with STD hamsters. Supplementation with spirulina, enriched or not, provides no protection against liver steatosis, even if the number of PMN cells was decreased. However, increases in cytokine levels were prevented in the SES group.

The theory proposed previously [36] suggested that in NASH, an amplification loop exists between IR and oxidative stress and results in overproduction of inflammatory cytokines. In the present study, the prevention of IR and the attenuated $O_2^{\circ -}$ production observed in the HF-SES group related to a lower production of inflammatory cytokines could traduce a protection against NASH induced by the HFD.

NF-κB, an oxidative stress-sensitive transcription factor, is a key regulator of expression of many genes involved in immune and inflammatory response, and NF-κB–induced cytokines widely contribute to the burden of inflammatory diseases, including atherosclerosis [37]. In our study, SES supplementation significantly prevented liver NF-κB activation induced by the HFD and the inherence of proinflammatory cytokines. The antioxidant effect of SES likely contributed to the inhibition of NF-κB activation.

Moreover, MCP-1 plays a critical role in the development of cardiovascular diseases. MCP-1, by its chemotactic activity, induces diapedesis of monocytes from the lumen to the subendothelial space, where they become foam cells, initiating fatty streak development that leads to atherosclerotic plaque formation [38]. Here, SES supplementation reduced the MCP-1 plasma level, suggesting that SES could limit the development of atherosclerosis. In addition to dyslipidemia, IR, oxidative stress, and inflammation, the HF diet is known to induce vascular dysfunction [25,39]. Indeed, in our model, we observed genuine dysfunction characterized by altered responses to contractile agonist and antagonist, and to endothelium-dependent vasorelaxant. SES supplementation efficiently prevented these alterations, these effects reflecting both avoidance of endothelial dysfunction and vascular remodeling known to be induced by the HFD [39]. No beneficial effect was observed when supplementation was performed with crude spirulina, suggesting that the preventive effects of SES on vascular dysfunction could be attributable to silicon enrichment.

In a previous work, it was reported that, after supply of oral soluble silica or coral sand in spontaneously hypertensive rats, blood pressure was reduced, and that this supply improved the related aortic gene expression [18]. In our study, using a different model and silicon supplementation through SES, we showed a prevention of vascular and endothelial functions assessed respectively by the contractile response to the agonist phenylephrine and the relaxation induced by Ach. This could corroborate earlier results [18].

Another study showed that rabbits given intravenous or oral silicon, at different doses and chemical form than that used here, had a reduction in the size of atherosclerotic plaques [17]. Using a different model and a different supply mode makes comparison difficult. However, our results strengthen previous findings [17]

and in addition, we have shown positive effects on IR, NAFLD, vascular reactivity, and inflammation.

Conclusion

The results of this study demonstrated that, in addition to the intrinsic nutritional advantages of spirulina, SES supplementation had genuine benefits, especially on IR and vascular function. The study revealed the efficiency of silicon in synergy with spirulina in a diet-induced hamster model of early atherosclerosis where SES prevented metabolic, oxidative, inflammatory disorders and vascular dysfunction. These properties open interesting perspectives in the context of silicon deficiency where intake is sufficient but bioavailability limited.

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